

CHROMOSOMES OF NEOTROPICAL RUBIACEAE. I: RUBIOIDEAE¹

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ABSTRACT

This paper presents chromosome data for 130 accessions of 91 species or subspecific taxa representing 26 genera of Neotropical Rubioideae (Rubiaceae). It includes the first chromosome counts for the tribe Perameae, for 11 genera, and for 71 species and subspecific taxa. A survey of the karyological knowledge (chromosome numbers, ploidy levels, chromosome morphology) for Neotropical Rubioideae is given. The relevance of the karyological information for a discussion on relationships and speciation in the Rubioideae is commented on. Possible taxonomic implications of the data are presented.

Key words: Chromosome numbers, karyology, Neotropics, Rubiaceae, Rubioideae.

In the course of comprehensive karyosystematic studies on Rubiaceae (Kiehn, 1986, 1995), existing data for Neotropical members of the family were compiled and numerous new counts were made. Up to now, karyological information on New World Rubiaceae has been very limited, with the few published data scattered in the literature. Based on detailed literature studies (especially new molecular phylogenetic approaches) and the new chromosome data, implications for an understanding of radiation, adaptation, evolution, and systematics of the investigated groups of the Rubioideae are discussed. A second paper (Kiehn, in prep.) will explore the other representatives of the Rubiaceae, recently united in one subfamily (Cinchonoideae) by Robbrecht and Manen (2006).

MATERIALS AND METHODS

Karyological investigations are based on field fixations or fixations from plants cultivated at the Botanical Garden of the University of Vienna, Vienna, Austria (HBV). Fixations of meristematic tissues (actively growing root tips, young flowers or apices for counts of mitotic numbers, young flower buds for meiotic investigations) were made in a freshly mixed 3:1 solution of ethanol (96%):glacial acetic acid. In most cases, fixations made from germinating seeds were pretreated with 0.002 M 8-hydroxyquinoline solution for 6 hr. at 8°C–10°C in the dark (see

Table 1). The fixations were kept under cool conditions at approximately 8°C. Chromosome staining was performed with Feulgen reagent, Giemsa, or acetocarmine (see Kiehn et al., 1991; Kiehn, 1995, for details on staining procedures). Collection data, tissues investigated, and techniques used for each species investigated are listed in Table 1. Permanent slides for the counts are deposited in the personal collection of the author. Voucher specimens have been deposited in the herbarium of Vienna University (WU) and/or in other herbaria as stated in Table 1.

The tribal arrangement of the Rubioideae in this paper follows Robbrecht and Manen (2006).

RESULTS AND DISCUSSION

Table 1 presents the new chromosome data and, when applicable, also includes references to earlier counts. Data for 91 species and subspecific taxa (130 accessions) of 26 genera are reported. They include the first chromosome number reports for the tribe Perameae, for 11 genera, and for 71 species. Data for 15 species are in accord with earlier reports; five reports deviate from earlier counts.

BASAL RUBIOIDEAE SENSU ROBBRECHT AND MANEN (2006)

Perameae and *Lasiantheae*. The monogeneric tribe *Perameae* was separated from *Spermacoceae* by

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Table 1. Chromosome numbers of Neotropical Rubiaceae–Rubioidae.

Taxon	Voucher	<i>n</i>	<i>2n</i>	PL	P	St	Ti	Former counts; remarks and comments
Basal Rubioideae sensu Robbrecht and Manen (2006)								
Perameae Bremek. ex S. P. Darwin								first counts for the tribe and the genus
<i>Perama</i> Aubl.								
<i>P. hirsuta</i> Aubl.	Ehrendorfer 7800-4208	—	18*	2x	—	A	ov	
	Gottsberger 16-2267	—	72 ± 2*	8x	—	A	ov	
Lasiantheae B. Bremer & Manen								first count for a Neotropical member of the tribe
<i>Ronabea</i> Aubl.								first count for the genus
<i>R. latifolia</i> Aubl.	Kiehn MK 961117-1/6	—	44-48*	4x	—	F	ov	= <i>Psychotria erecta</i> (Aubl.) Standl. & Steyerl.
Coussareeae Hook. f., sensu Bremer and Manen (2000)								including Coccocypseae Bremek., Cruckshanksiae Hook. f.
<i>Coccocypselum</i> P. Browne								formerly part of the Coccocypseae Bremek.; for earlier counts, see Kiehn (1989)
<i>C. brevipetiolatum</i> Steyerl.	Kiehn RR 1028	—	20	2x	H	F	rt	
<i>C. decumbens</i> K. Krause	Kiehn RR 756	—	20	2x	H	A	rt	
<i>C. guianense</i> (Aubl.) K. Schum.	Billiet 1860	—	40	4x	—	A	rt	
<i>C. herbaceum</i> P. Browne	Kiehn & Kiehn MK 300786-1/5	10	—	2x	—	F	PMC	
<i>C. hirsutum</i> Bartl. ex DC.	Kiehn 1983-1105	—	20	2x	H	A	rt	
	Kiehn 1983-1501	—	20 ± 2	2x	H	F	rt	
	Vogel et al. 1984-81-10/2	—	40	4x	H	F	rt	
<i>C. umbellatum</i> Poir.	Kiehn 1983-1499	—	38-40	4x	H	A	rt	
<i>Coccocypselum</i> sp. indet.	Kiehn 1986-1602	20	—	4x	—	F	PMC	
Coussarea Aubl.								first count for the genus
<i>C. congestiflora</i> Müll. Arg.	Ehrendorfer 74919-2-1	—	22*	2x	—	A	fl	
Cruckshanksia Hook. & Arn.								formerly part of the Cruckshanksiae Hook. f.; first count for the genus
<i>C. hymenodon</i> Hook. & Arn.	Grau 2522	—	22*	2x	—	F	rt	
Declieuxia Kunth								formerly part of the Psychotrieae Cham. & Schltld.; first count for the genus
<i>D. fruticosa</i> (Willd. ex Roem. & Schult.) Kuntze	Gottsberger 13-2267	18-20*	—	4x	—	F + A	PMC	
Faramea Aubl.								first counts for the genus
<i>F. glandulosa</i> Poepp. & Endl.	Huber & Weißenhofer 9917-1	—	22*	2x	—	F + G	ov, fi	count by M. Pass

Table 1. Continued.

Taxon	Voucher	<i>n</i>	<i>2n</i>	PL	P	St	Ti	Former counts; remarks and comments
<i>F. suerrensis</i> Donn. Sm.	<i>Crex</i> 9907-2	10–11*	22*	2x	—	F	PMC, ov	counts by M. Pass
	Heiselmayer 950220-2/1	10, 11*	22*	2x	—	F	PMC, ov	meiosis irregular, resulting in different haploid numbers
<i>F. tetragona</i> Müll. Arg. or aff.	Ehrendorfer & Gottsberger 73825-1324	—	18–22*	2x	—	A	ov	
Supertribe Psychotriidinae Robbr. & Manen								
Morindeae Miq.								
<i>Morinda</i> L.								
<i>M. panamensis</i> Seem.	<i>Crex</i> 9909-2	—	22*	2x	—	F + G	ap	count by M. Pass
Psychotrieae Cham. & Schldl.								
<i>Psychotria</i> L.								
<i>Psychotria</i> subg. <i>Psychotria</i>								
<i>P. carthagenensis</i> Jacq.	Kiehn RR-88-18	—	44*	4x	H	F	rt	
<i>P. graciliflora</i> Benth.	Kiehn & Kiehn MK 030485-3/3	—	22–24*	2x	—	A	fl	
<i>P. grandis</i> Sw.	Ehrendorfer 6400-2502	—	22–24*	2x	—	A	st	
<i>P. horizontalis</i> Sw.	Ehrendorfer 74108-4-9	—	22*	2x	—	F	fl	
<i>P. ligustrifolia</i> (Northr.) Millsp.	Eckhardt s.n.	—	22	2x	—	A	fi	Lewis (1962): <i>2n</i> = 22 (U.S.A.)
<i>P. marginata</i> Sw.	Ehrendorfer 6400-2903	11*	—	2x	—	A	PMC	
	Kiehn & Kiehn MK 200385-2/1	—	22–24*	2x	—	F	st	
	Kiehn & Kiehn MK 300786-1/3	—	22*	2x	—	F	st	
<i>P. parvifolia</i> Oerst.	Kiehn & Kiehn MK 880309-2/2	—	22*	2x	—	F	fl	
<i>P. sylvivaga</i> Standl.	Kiehn & Kiehn MK 030485-3/5	—	—	12–14x*	—	A	fl	
Palicoureeae Robbr. & Manen								
<i>Geophila</i> D. Don.								
<i>G. macropoda</i> (Ruiz & Pav.) DC.	Lindeman JCL 599	—	22*	2x	H	F	rt	
<i>G. repens</i> (L.) I. M. Johnst.	Zanoni et al. 15980	—	44	4x	H	F	rt	discussion of earlier counts: Hellmayr et al. (1994)
<i>Notopleura</i> (Benth. & Hook. f.) Bremek. (formerly <i>Psychotria</i> subg. <i>Heteropsychotria</i> sect. <i>Notopleura</i>)								first counts for the genus
<i>Notopleura</i> subg. <i>Notopleura</i>								
<i>N. anomothyrsa</i> (K. Schum. & Donn. Sm.) C. M. Taylor	<i>Crex</i> 9915-2	11*	22*	2x	—	F	PMC, ov	
<i>N. capacifolia</i> (Dwyer) C. M. Taylor	Kiehn & Kiehn MK 880316-1/4	44, 55*	—	8x, 10x	—	F	PMC	different numbers in pollen mitoses in different flowers

Table 1. Continued.

Taxon	Voucher	n	2n	PL	P	St	Ti	Former counts; remarks and comments
<i>N. polyphlebia</i> (Donn. Sm.) C. M. Taylor	Kiehn MK 961117-1/2	—	44*	4x	H	F + C	rt	
	Kiehn MK 961118-2/1	—	44*	4x	H	F + G	rt	
<i>N. uliginosa</i> (Sw.) Bremek.	Kiehn & Kiehn MK 880309-1/4	22*	—	4x	—	F + A	PMC	
	Heiselmayer 950212-1/1	—	44*	4x	H	F	rt	
<i>Notopleura</i> subg. <i>Viscagoga</i> (Baill.) C. M. Taylor								
<i>N. pithecobia</i> (Standl.) C. M. Taylor	Kiehn MK 950217-1/2	—	44*	4x	H	F + G	rt	
<i>Palicourea</i> Aubl.								
<i>P. alpina</i> (Sw.) DC.	Taylor 7076	—	40–44*	4x	—	F	fl	
	Taylor 7077	—	40–44*	4x	—	F	fl	
<i>P. blanchetiana</i> Schltdl.	Ehrendorfer 74922-1-6	—	22*	2x	—	A	ov	
<i>P. crocea</i> (Sw.) Roem. & Schult.	Till 18017	—	22*	2x	H	F	rt	
	Taylor 6935	11*	—	2x	—	F	PMC	
	Taylor 7084	11*	—	2x	—	F	PMC	
	Taylor 7087	11*	—	2x	—	F	PMC	
<i>P. croceoides</i> Ham.	Taylor 6938	—	64–66*	6x	—	F	fi	
	Till 16063	—	64–66*	6x	—	G	fl	
<i>P. grandiflora</i> (Kunth) Standl.	Ehrendorfer 74922-1-4	—	22*	2x	—	A	ov	
<i>P. guianensis</i> Aubl.	Till 16015	—	22*	2x	—	G	fl	
<i>P. lasiorrhachis</i> Oerst.	Kiehn & Kiehn MK 880309-2/1	22*	44*	4x	—	F	PMC, ov	
<i>P. leuconeura</i> Standl.	Ehrendorfer 6400-4003	—	22*	2x	—	A	fi	
<i>P. nitidella</i> (Müll. Arg.) Standl.	Ehrendorfer 74913-6-3	—	22*	2x	—	A	ov	
<i>P. padifolia</i> (Willd. ex Roem. & Schult.) C. M. Taylor & Lorence	Kiehn & Kiehn MK 030485-1/1	22*	44*	4x	—	F	PMC, ov	
	Kiehn & Kiehn MK 880319-1/8	22*	44*	4x	—	F	PMC, ov	
	Vogel et al. 1984-87-2/1	—	44*	4x	—	F	rt	
<i>P. quadrifolia</i> (Rudge) DC.	Ehrendorfer 74922-1-5	—	22*	2x	—	F	fl	
<i>Psychotria</i>								for earlier counts for the Neotropics, see text
<i>Psychotria</i> subg. <i>Heteropsychotria</i> Steyerm.								
<i>P. aubletiana</i> Steyerm. var. <i>andina</i> Steyerm.	Ehrendorfer 6400-5009	55–59*	—	10x	—	F	PMC	
<i>P. aubletiana</i> var. <i>aubletiana</i>	Kiehn & Kiehn MK 880319-1/2	33*	—	6x	—	F	PMC	
	Kiehn et al. MK 880321-1/5	—	64–66*	6x	—	F	fl	
	Kiehn & Kiehn MK 880309-2/5	33*	66*	6x	—	F	PMC, ov	
<i>P. berteriana</i> DC.	Ehrendorfer 7900-46-1	—	44 ± 1*	4x	—	A	st	
<i>P. buchtienii</i> (H. J. P. Winkl.) Standl.	Kiehn MK 961119-2/1	—	44*	4x	—	F	ov	= <i>P. officinalis</i> (Aubl.) Raeusch. ex Sandwith

Table 1. Continued.

Taxon	Voucher	n	2n	PL	P	St	Ti	Former counts; remarks and comments
<i>P. capitata</i> Ruiz & Pav.	Till 16058	—	22*	2x	—	G	fl	
	Kiehn MK 961118-4/1	—	44*	4x	H	F + G	rt	
<i>Psychotria</i> cf. <i>cephalantha</i> (Müll. Arg.) Standl.	Ehrendorfer & Gottsberger 73825-1319	—	88 ± 4*	8x	—	A	fl	
<i>P. chiriquiensis</i> (Standl.) C. M. Taylor	Kiehn et al. MK 880321-1/4	—	44*	4x	H	F	rt	
<i>P. cooperi</i> Standl.	Kiehn & Kiehn MK 880309-1/1	22*	44*	4x	H	F	PMC, ov	
<i>P. cyanococca</i> Seem. ex Dombrain	Kiehn & Kiehn MK 190385-2/1	—	22*	2x	H	F	rt	= <i>P. pittieri</i> Standl.
	Till & Till s.n.	—	22*	2x	H	F	rt	
<i>P. elata</i> (Sw.) Hammel or <i>P. borucana</i> (Ant. Molina) C. M. Taylor & W. C. Burger	Kiehn MK 961119-3/6	—	44*	4x	H	F + G	rt	
<i>P. elata</i> (Sw.) Hammel	Kiehn & Kiehn MK 880309-1/3	—	44*	4x	H	F	rt	
<i>P. furcata</i> DC.	Kiehn & Kiehn MK 200385-2/5	—	22*	2x	—	A	fl	
<i>P. hazenii</i> Standl.	Kiehn & Kiehn MK 880316-1/3	—	22*	2x	—	F	fl	originally determined as <i>P. ramonensis</i> Standl.
	Heiselmayer 950217-1/1	—	44*	4x	—	F	ov	
<i>P. hoffmannseggiana</i> (Willd. ex Roem. & Schult.) Müll. Arg.	Ehrendorfer 74922-2-9	22 ± 2*	—	4x	—	A	PMC	Pinto-Maglio et al. (1997): 2n = 22
<i>P. iodotricha</i> Müll. Arg.	Ehrendorfer 74915-1-16	—	22*	2x	—	A	fl	
<i>P. leuocarpa</i> Cham. & Schltdl.	Ehrendorfer 7300-4003	—	44*	4x	—	A	fl	
	Ehrendorfer 7300-4004	—	44*	4x	—	A	fl	
<i>P. longipes</i> Müll. Arg.	Gottsberger 15-241066	66 ± 1*	—	12x	—	F + A	fl	
<i>P. lupulina</i> Benth. subsp. <i>rhodoleuca</i> (Müll. Arg.) Steyerf.	Ehrendorfer 74913-1-6	22*	—	4x	—	A	PMC	meiosis in flower buds < 2 mm
<i>P. mima</i> Standl.	Ehrendorfer 6400-5409	—	22 ± 2*	2x	—	A	fl	
<i>P. phanaerandra</i> (Standl. & Steyerf.) Lorence	Kiehn & Kiehn MK 880309-1/11	—	40-44*	4x	—	F	ov	= <i>P. luteotuba</i> Lorence
<i>P. pilosa</i> Ruiz & Pav.	Bernhard & Greger HG2607086	—	22*	2x	—	G + A	ap	
<i>P. poeppigiana</i> Müll. Arg. subsp. <i>barcellata</i> (Müll. Arg.) Steyerf.	Ehrendorfer 74104-1-9	—	44 ± 2*	4x	—	A	fl	
<i>P. poeppigiana</i> Müll. Arg. subsp. <i>poeppigiana</i>	Kiehn & Kiehn MK 090886-2/3	—	44*	4x	H	F	rt	
<i>P. solidum</i> Standl.	Bernhard & Greger HG2607083	—	44*	4x	—	G + A	ap	
	Kiehn MK 961117-1/4	—	44*	4x	—	F + G	ov	
	Kiehn MK 961118-4/2	22*	42-44*	4x	—	F	PMC, ov	
<i>P. suerrensii</i> Donn. Sm.	Kiehn & Kiehn MK 880309-1/7	—	44 ± 2*	4x	—	F	fl	

Table 1. Continued.

Taxon	Voucher	<i>n</i>	2 <i>n</i>	PL	P	St	Ti	Former counts; remarks and comments
<i>P. tepuiensis</i> (Steyerm.) Steyerm.	Till 16059	—	44 ± 2*	4x	—	G	fl	
<i>Psychotria</i> sp. indet. aff. <i>P. mima</i> Standl.	Ehrendorfer & Gottsberger 73825-13-12	—	44*	4x	—	A	fi	
<i>Psychotria</i> sp. indet.	Ehrendorfer 74927-1-13	—	22*	2x	—	A	fl	
Psychotria collections not assigned to a subgenus								
<i>Psychotria</i> sp. indet.	Ehrendorfer 74915-1-17	—	22*	2x	—	A	fl	
<i>Psychotria</i> sp. indet.	Ehrendorfer 6400- 5005	22*	—	4x	—	A	PMC	
<i>Rudgea</i> Salisb.								first count for the genus
<i>Rudgea</i> sp. indet	Gottsberger 18-241066	66 ± 1*	—	12x	—	A	po	
Supertribe Rubiidae Robbr. & Manen—Rubiidae I sensu Robbrecht and Manen (2006)								
Anthospermeae Cham. & Schltdl.								
<i>Nertera</i> Banks & Sol. ex Gaertn.								
<i>N. depressa</i> Banks & Sol. ex Gaertn.	Frimmel 8-90		44	4x	H	G	rt	= <i>N. granadensis</i> (Mutis ex L. f.) Druce; for earlier counts, see Kiehn (1996), Kiehn et al. (2005)
Rubieae Baill.								
<i>Didymaea</i> Hook. f.								first count for the genus
<i>D. mexicana</i> Hook. f. ¹	Kiehn et al. MK 920211-2/2	—	22*	2x	H	F	rt	
<i>Galium</i> L.								
<i>G. hypocarpium</i> (L.) Endl. ex Griseb.	Kiehn & Kiehn MK 090886-2/6	—	44 ^a	4x	—	F + G	rt	Huynh (1965): <i>n</i> = 33 (Peru); Kiehn et al. (2005): 2 <i>n</i> = 22 (Chile)
Supertribe Rubiidae Robbr. & Manen—Rubiidae II sensu Robbrecht and Manen (2006)								
Spermacoceae Bercht. & J. Presl sensu Robbrecht and Manen (2006)								including Hedyotideae Cham. & Schltdl. p.p., Manettieae Bremek.
<i>Arcytophyllum</i> Roem. & Schult.								
<i>A. lavarum</i> J. D. Smith	Crex 9913-4	—	36*	4x	—	F	ov	
	Kiehn & Kiehn MK 880311-3/1	—	36*	4x	—	F	ov	
<i>Bouvardia</i> Salisb.								for synonyms, see revision of Blackwell (1968)
<i>B. laevis</i> M. Martens & Galeotti	Balls 5465	—	34–36*	4x	—	F	ap	
	Beyer 166	18*	—	4x	—	A	PMC	
<i>B. ternifolia</i> (Cav.) Schltdl.	Vogel et al. 1984-2-12/2	—	72	8x	—	F	rt	Lewis (1962): <i>B. ternifolia</i> (Cav.) Schltdl. (partly as <i>B. scabrata</i> M. Martens & Galeotti): <i>n</i> = 18 (Mexico), <i>n</i> = 36 (Mexico, U.S.A.), <i>n</i> = 54 (Mexico)
	Boutin 3956	—	102–110	12x	—	F	rt	
	Ehrendorfer 7900-57-1	54	—	12x	—	A	PMC	

Table 1. Continued.

Taxon	Voucher	n	2n	PL	P	St	Ti	Former counts; remarks and comments
<i>Diodia</i> L.								
<i>D. rigida</i> Cham. & Schltdl.	Heitselmayer 950215-3/1	—	56†	4x	H	F + G	n	= <i>Diodia opiculata</i> (Willd. ex Roem. & Schult.) K. Schum. first count for the genus
<i>Emmeorrhiza</i> Pohl ex Endl.								
<i>E. umbellata</i> (Spreng.) K. Schum.	Ehrendorfer & Gottsberger 73821-3-5	—	28*	2x	—	A	fl	first count for the genus
<i>Ernodea</i> Sw. ²								
<i>E. cokeri</i> Britton ex Coker	Negrón-Ortiz s.n. (1)	—	28	2x	—	F	fl	definitely not more than 2n = 30
<i>E. littoralis</i> Sw.	Negrón-Ortiz & Pinder 335, 336	—	—	2x	—	F	fl	definitely not more than 2n = 30
<i>E. mullspanghi</i> Britton	Kiehn RR 87-21	—	28	2x	H	F	n	chromosomes
	Negrón-Ortiz s.n. (2)	—	24-30	2x	—	F	fl	
	Negrón-Ortiz s.n. (3)	—	—	2x	—	F	fl	definitely not more than 2n = 30
<i>Hemidiodia</i> K. Schum.								
<i>H. ocyμφolia</i> (Willd. ex Roem. & Schult.) Kiehn et al. MK 880321-1/3		—	28*	2x	H	F + G	n	first count for the genus; incorporated in <i>Borreria</i> subg. <i>Dasycephalata</i> (DC.) Bacigalupo & E. L. Cabral by Bacigalupo and Cabral (1996) = <i>Borreria ocyμφolia</i> (Roem. & Schult.) Bacigalupo & Cabral = <i>Diodia ocyμφolia</i> (Roem. & Schult.) Bremek.
<i>Manettia</i> Mutis ex L.								
<i>M. barbata</i> Oerst.	<i>Crex</i> 9906-1	—	22*	2x	—	F	fl	
<i>M. luteo-rubra</i> (Vell.) Benth. var.	Kiehn RR 95-04	22*	—	4x	—	A	PMC	Fagerlind (1937), as <i>M. inflata</i> Sprague; 2n < 40 (cult.); Pouques (1949): <i>Manettia luteo-rubra</i> (Vell.) Benth. var. <i>luteo-rubra</i> (as <i>M. bicolor</i> Paxton); 2n = 22 (cult.) numerous counts on diploid and tetraploid levels, see Kiehn (1985)
<i>Oldenlandia</i> L.								
<i>O. corymbosa</i> L.	Kiehn & Kiehn MK 270786-1/1	—	18	2x	H	F	n	
<i>Richardia</i> L.								
<i>R. brasiliensis</i> Gomes	Kiehn RR 663	—	28	2x	H	F	n	Lewis (1966): n = 14 (U.S.A., Brazil, South Africa; Lewis (1962): n = 42 (U.S.A.)
<i>R. scabra</i> L.	Kiehn & Kiehn MK 260786-1/1 Kiehn & Kiehn MK 290385-1/2 Kiehn & Kiehn MK 200886-1/1 Kiehn & Kiehn MK 880331-1/1	— — — —	56 56 56 56	4x 4x 4x 4x	H H H H	F + G F F F	n n n n	Lewis (1962): n = 28; Philip and Mathew (1988): n = 14

Table 1. Continued.

Taxon	Voucher	n	2n	PL	P	St	Ti	Former counts; remarks and comments
<i>Richardia</i> sp. indet. Spermacoce L.	<i>Forstreuter s.n.</i>	—	28	2x	H	F	n	including <i>Borreria</i> sensu Bacigalupo and Cabral (1996); excluding <i>Hemidiodia</i> (see above)
<i>S. assurgens</i> Ruiz & Pav.	Kiehn & Kiehn MK 160385-1/1	—	28*	2x	H	F	n	= <i>Borreria remota</i> (Lam.) Bacigalupo & Cabral
<i>S. confusa</i> Rendle	Kiehn & Kiehn MK-050886-1/2	—	28*	2x	H	F	n	
	Kiehn & Kiehn MK 250385-1/2	—	28*	2x	H	F	n	
<i>S. ocyroides</i> Burm. f.	Kiehn & Kiehn MK 210385-1/9	—	52-56*	4x	H	F	n	= <i>Borreria ocyroides</i> (Burm. f.) DC.
"Spermacoceae" sp. indet.	Ehrensdorfer & Gotsberger 73822-602	14	—	2x	—	A	PMC	available literature did not lead to satisfying identification
	Gotsberger 11-1267	—	28	2x	—	A	n	
	Gotsberger 15-2267	—	28	2x	—	A	n	

* n, haploid chromosome number; 2n, diploid chromosome number; PL, ploidy level; P, pretreatment; St, staining method; Ti, tissue. Asterisks (*) represent the first count for the genus; † indicates the first count for the species; ‡ indicates deviation from literature data. Pretreatment and staining methods are indicated by: H, 8-hydroxyquinoline; A, acetocarmine; F, Feulgen reagent; G, Giemsa. Source material of counts is indicated as: PMC, pollen mother cell; fl, young flower bud; ov, young ovary; st, stylar tissue; fl, filament tissue; r, root tips; ap, shoot apex; po, pollen mitosis. Detailed collecting data are presented in Appendix 1.

† According to unpublished studies by D. H. Lorence (pers. comm.), the current assignment of the name *Didymaea alsinoides* to the Central American taxon of this genus is incorrect; the correct name must be *D. mexicana*.

‡ In the course of the present studies, exact chromosome numbers could only be detected for *Ernodea cokeri* from the Bahamas and *E. littoralis* from Cuba. Due to the quality of the field fixations and the large size of the chromosomes (ca. 4 µm in mitotic prometaphase), all other counts either revealed a potential chromosome number range or it was only possible to establish the ploidy levels. The results were, as reported here in detail, communicated to Negrón-Ortiz. They were cited by Negrón-Ortiz (1996: 403) as "... the same basic chromosome number, n = 14, is exhibited by the currently recognized taxa (*E. cokeri* Britton ex Coker, *E. littoralis*, *E. millspaughii* Britton, and *E. taylori* Britton; Kiehn, pers. comm.)." and in Negrón-Ortiz and Hickey (1996: 454) as "... the basic chromosome number, x = 14, does not vary among *E. cokeri* (Great Abaco), *E. littoralis* (Cuba and Great Abaco), *E. millspaughii* (Great Inagua), and *E. taylori* (Great Inagua) (Kiehn, pers. comm.)." These statements could be correct, but do not accurately reflect the data communicated to Negrón-Ortiz.

Bremekamp (1966), based on earlier observations (Bremekamp, 1952: footnote on pp. 13–14). The first karyological investigations on members of the genus *Perama* Aubl. revealed $x = 9$ as the basic number for the genus. The two counted populations of *P. hirsuta* Aubl. show different ploidy levels ($2x$ and $8x$). Because this species comprises several varieties (Steyemark, 1974), more counts would be desirable to elucidate potential correlations between subspecific units and ploidy levels.

The Lasiantheae were established by Bremer and Manen (2000) and are proposed to comprise the genera *Lasianthus* Jack, *Ronabea* Aubl., *Saldinia* A. Rich. ex DC., and *Trichostachys* Hook. f. by Robbrecht and Manen (2006). The first count for a species of the genus *Ronabea*, resurrected by Taylor (2004), reveals the tetraploid level for *R. latifolia* Aubl. (formerly *Psychotria erecta* (Aubl.) Standl. & Steyerl.). No other chromosome numbers for Neotropical members of tribe Lasiantheae are known.

In the *rps16* intron analysis of Andersson and Rova (1999), the genus *Perama* appears sister to *Lasianthus* at a basal node of the Rubioideae phylogeny. The Lasiantheae form the third basal clade (after Ophiorrhizeae and Urophylleae) in the strict consensus tree for the Rubioideae of Bremer and Manen (2000); this study did not include *Perama* due to the lack of material. The supertree of Robbrecht and Manen (2006) shows *Perama* and *Lasianthus* as one of two clades in a grade at the base of the Coussareeae. Do the cytological results corroborate the relationships of the Perameae and the Lasiantheae suggested by these molecular studies?

The present count for *Ronabea* indicates a basic number of $x = 11$ or $x = 12$ on the tetraploid level. Additional data for Lasiantheae are rare: for the genus *Lasianthus*, there is only one published count of an Old World species (*L. acuminatus* Wight from India, $n = 44$; Bir et al., 1984) indicating octoploidy and $x = 11$ as the basic number. Several attempts made by the author to get additional chromosome data for *Lasianthus* resulted in only one approximate count (for *L. lancilimbus* Merr. from China, $2n > 200$; Kiehn, unpublished data); this was due to the presence of tannins in the fixations causing dark chromosomes that clumped together. The field fixations of *Ronabea* (as well as cytological fixations of *Saldinia* material from Madagascar; Kiehn, unpublished data) exhibited the same effects of tannins, while the two investigated *Perama* field fixations did not show such features. Thus, neither basic number nor chemical compounds interacting with chromosome fixatives support the assumption of a close relationship between *Perama* and Lasiantheae.

How can the cytological results for *Perama* be interpreted in relation to other Rubioideae? Chromosomes of *Perama* and the Spermacoceae have different staining patterns with acetocarmine (*Perama*: stains well, with no cytoplasmatic staining; Spermacoceae: contrast of chromosomes against cytoplasm is weak); they are also different in condensation of the chromosomes (see Kiehn, 1995: all Spermacoceae exhibit late condensation of telomeric parts of the chromosomes, which is unusual in Rubiaceae and not found in *Perama*). Thus, a position of *Perama* near to Spermacoceae is not supported by chromosome characteristics. Of all groups in closer proximity to *Perama* in the Rubioideae supertree of Robbrecht and Manen (2006: fig. 7), only the Urophylleae s.l. exhibit $x = 9$ and relatively small *Perama*-like chromosomes (Kiehn, 1995). However, the *Pauridiantha* Hook. f. and *Urophyllyum* Wall. species investigated so far exhibit a bimodal karyotype (Kiehn, 1985, 1995), a situation that could not be documented for the two *Perama* accessions studied here. Thus, the presently known cytological findings give no further indication about relationships of the Perameae.

Coussareeae. The tribe Coussareeae as emended by Bremer and Manen (2000) comprises taxa formerly included in several tribes (Coussareeae, Coccocypseleae, and Cruckshanksieae) by Robbrecht (1988) and, in the supertree of Robbrecht and Manen (2006), is placed as sister group to the Psychotriidinae and the Rubioidinae.

Only chromosome numbers for the genus *Coccocypselum* P. Browne were reported so far (Kiehn, 1989, conference abstract). The unpublished collection details for the reports of Kiehn (1989) and an additional count for *C. hirsutum* Bartl. ex DC. are included in Table 1. Based on these data for seven taxa, *Coccocypselum* exhibits a basic number of $x = 10$ with taxa on the diploid and tetraploid ploidy level. In *C. hirsutum*, the occurrence of two cytotypes (diploid and tetraploid) is documented.

The first chromosome counts for the genera *Coussarea* Aubl., *Cruckshanksia* Hook. & Arn., *Declieuxia* Kunth, and *Faramea* Aubl. are presented here. The investigated species of *Coussarea* and *Cruckshanksia* are diploids on $x = 11$ (one species of each genus). In *Faramea*, three species (four accessions) were counted; all are diploids. Regarding basic numbers, the obtained picture is not clear. One species (*F. glandulosa* Poepp. & Endl.) shows $x = 11$ (counts on mitotic divisions). In *F. suerrensii* Donn. Sm., all counts on somatic tissues for two accessions also revealed $2n = 22$. However, meiotic irregularities were observed, leading to gametes with $n = 10$ as well as $n = 11$. In the third investigated species (*F.*

tetragona Müll. Arg.), the basic number could not be established with certainty. Based on these data, $x = 11$ can be assumed to be the "regular" basic number in *Faramaea*. The only counted *Declieuxia* species, *D. fruticosa* (Willd. ex Roem. & Schult.) Kuntze, is tetraploid on either $x = 9$ or $x = 10$ as the basic number. This result concurs with the proposed exclusion of *Declieuxia* from the Psychotrieae (Bremer & Manen, 2000), where $x = 11$ is by far the predominant basic number (Kiehn, 1995; see also discussion below). It also fits with the palynological findings presented by Piesschaert et al. (1999) stating a distinct pollen type shared by *Coccocypselum*, *Declieuxia*, and *Hindsia* Benth. (the latter not known cytologically yet), and with the molecular data (nuclear DNA [nDNA] and chloroplast DNA [cpDNA]; Andersson & Rova, 1999; Bremer & Manen, 2000; Robbrecht & Manen, 2006), where *Coccocypselum* and *Declieuxia* form parts of a well-supported clade.

In summary, it can be stated that the tribe Coussareae of Bremer and Manen (2000) is cytologically heterogeneous. In terms of phylogenetic trends, an ascending dysploid series might be a possible explanation if it is assumed that the relationships expressed by Andersson and Rova (1999) are correct. The tribe then has $x = 11$ as the original basic number, present in the more basal *Cruckshanksia* and in the molecularly well-supported clade consisting of *Faramaea* and *Coussarea*, while the clade formed by *Coccocypselum* and *Declieuxia* shows a derived status of $x = 10$ (or even $x = 9$). The most probable hypothesis for explaining the observations is that several parallel changes of basic chromosome numbers occurred in the different genera. This would also best explain the situation documented for *Faramaea*.

SUPERTRIBE PSYCHOTRIIDINAE

Morindeae. Only few chromosome data exist for members of this tribe, and all exhibit $x = 11$. The statistical data for *Morindeae* given by Kiehn (1995) need to be adjusted, as the genus *Lasianthus*, which is responsible for the reports of the high ploidy levels ($8x$ and $20-22x$) in Kiehn (1995), is excluded from the *Morindeae* (Bremer & Manen, 2000) and transferred to the Lasiantheae. The remaining counted taxa of *Morindeae* are either diploids or tetraploids.

In the Neotropics, only one taxon has been investigated cytologically so far. *Morinda royoc* L. is diploid ($n = 11$; Lewis, 1962; $2n = 22$; Fritsch, 1970). For the pantropical *M. citrifolia* L., all counts (revealing one diploid and several tetraploid results) were carried out on accessions from outside the New World (see Kiehn, 1985). The new count for *M.*

panamensis Seem. ($2n = 22$) fits well into the general cytological picture of the genus. Chromosome structure and staining behavior of *Morinda* L. are similar to the patterns found in *Psychotria* L., especially in subgenus *Psychotria*.

Psychotrieae s.l. Members of Psychotrieae s.l. (Psychotrieae s. str. sensu Robbrecht & Manen, 2006, and Palicoureeae Robbrecht & Manen, 2006) are found in the tropics and subtropics worldwide. In the Neotropics, the tribe is represented by ca. 1000 species (Taylor, 1996). The largest genera in the region are *Psychotria* s.l. (ca. 600 species), *Palicourea* Aubl. (ca. 200), and *Rudgea* Salisb. (ca. 120) (Taylor, 1996).

Despite the huge number of Psychotrieae s.l. species in the Neotropics, up to now chromosome numbers have been reported for only eight members of the tribe from this region: five for taxa of *Psychotria* subg. *Heteropsychotria* Steyerl. and one for a species of *Psychotria* subg. *Psychotria*, *Carapichea* Aubl., and *Palicourea*, respectively. These reports all revealed a basic number of $x = 11$ (the number common for the bulk of the cytologically investigated Psychotrieae species worldwide; see Kiehn, 1995). For seven taxa, the reports are exclusively on the diploid level: *Palicourea marcgravii* A. St.-Hil. (Pinto-Maglio et al., 1997), *Psychotria brachyceras* Müll. Arg. (Kiehn & Lorence, 1996), *P. brasiliensis* Vell. (Fagerlind, 1937, as *P. nuda* Wawra), *P. erythrocarpa* Schldtl. (Kiehn & Lorence, 1996), *P. hoffmannseggiana* (Willd. ex Roem. & Schult.) Müll. Arg. (Pinto-Maglio et al., 1997), *P. ligustrifolia* (Northr.) Millsp. (Lewis, 1962), and *P. nervosa* Sw. (Lewis, 1962; Fritsch, 1970, both as *P. undata* Jacq.). One has diploid and tetraploid reports (*Carapichea ipecacuanha* (Brot.) L. Andersson = *Psychotria ipecacuanha* (Brot.) Stokes; Fagerlind, 1937; Janaki-Ammal, 1945). In *Palicourea marcgravii*, Pinto-Maglio et al. (1997) observed three additional (B-type) chromosomes.

The circumscription of the Psychotrieae s.l. (see Robbrecht, 1988; Taylor, 1996) has only recently been altered considerably. Several papers (see Robbrecht & Manen, 2006, for a survey) provided evidence that the Psychotrieae s.l. disintegrate into two clades. On the generic level, Nepokroeff et al. (1999) clearly showed that *Psychotria* in the traditional sense is paraphyletic. One consequence is that the monophyletic *Psychotria* sect. *Notopleura* Benth. & Hook. f. was reestablished at genus level (*Notopleura* (Benth. & Hook. f.) Bremek.) by Taylor (2001). Taylor (2004) excluded *Ronabea* from *Psychotria* and placed it into the Lasiantheae of Bremer and Manen (2000). Andersson (2002) subdivided the Psychotrieae into two major clades: the *Psychotria*

Table 2. Comparison of frequency of ploidy levels in the investigated Neotropical taxa of *Psychotria* s.l. (including information from literature).

	2x	4x	6x	8x	10x	12x(-14x)
<i>Psychotria</i> subgen. <i>Heteropsychotria</i> (31 taxa, three with two ploidy levels)	12 (35.3%)	18 (52.9%)	1 (2.9%)	1 (2.9%)	1 (2.9%)	1 (2.9%)
<i>Psychotria</i> subgen. <i>Psychotria</i> (10 taxa)	7 (70.0%)	2 (20.0%)	0	0	0	1 (10.0%)

complex and the *Palicourea* complex, the latter including *Carapichea*, *Geophila* D. Don, *Notopleura*, *Rudgea*, and an expanded *Palicourea* (including *Psychotria* subgen. *Heteropsychotria*). Both groups were formally circumscribed by Robbrecht and Manen (2006) as tribes Psychotrieae s. str. (in the Neotropics only comprising members of *Psychotria* subgen. *Psychotria*) and Palicoureeae (with the genera *Carapichea*, *Geophila*, *Palicourea*, *Margaritopsis* C. Wright, *Notopleura*, *Rudgea*, and *Psychotria* subgen. *Heteropsychotria* occurring in the Neotropics).

Psychotrieae s. str. In the present study, 10 taxa (12 origins) of *Psychotria* subgen. *Psychotria* are investigated, nine of them for the first time. Seven taxa investigated were diploids, two were tetraploids, and only one (*P. sylvivaga* Standl.) was a high polyploid. This is in marked contrast to the proportion of diploids to polyploids found in *Psychotria* subgen. *Heteropsychotria* (see Table 2).

Palicoureeae. Counts for 63 accessions of 46 taxa out of five genera of the Palicoureeae are presented here, including first generic reports for *Notopleura* and *Rudgea*.

All investigated genera have $x = 11$ as the basic number and show polyploidy. In *Geophila*, species on diploid and tetraploid levels are reported. The only count for *Rudgea* is on the 12x level. *Palicourea* species exhibit either 2x, 4x, or 6x. *Notopleura* species have taxa on 2x, 4x, and 8–10x. In all these genera, different accessions of the same species always have the same ploidy level. No indications for B chromosomes (as reported in *Palicourea* by Pinto-Maglio et al., 1997) could be found.

In *Psychotria* subgen. *Heteropsychotria*, taxa with 2x, 4x, 6x, 8x, 10x, or 12x are known now. *Psychotria* subgen. *Heteropsychotria* also comprises all four Neotropical *Psychotria* cases of different ploidy levels reported for different accessions of the same species. For *P. capitata* Ruiz & Pav., *P. hazenii* Standl., and *P. hoffmannseggiana*, both diploidy and tetraploidy are reported, and in the high polyploid *P. aubletiana* Steyermark there are counts on 6x (*P. aubletiana* var. *aubletiana*) and 10x (*P. aubletiana* var. *andina*

Steyermark.). The existence of different ploidy levels can be interpreted in several ways: it could be an indication of shortcomings in identifications (possibly as a result of the high number of *Psychotria* taxa described in the region). The different ploidy levels could also indicate the presence of two cytologically different units currently treated under one name (possibly the case in *P. aubletiana*, *P. capitata*, and *P. hoffmannseggiana*, as all of these are species with large distribution areas in Central America, the northern part of South America, and the Caribbean, with several subspecific entities recognized by Steyermark [1972, 1974]). In these cases, ploidy levels could provide additional arguments and characters for distinguishing and naming naturally existing taxa. The detected differences in ploidy levels also could be the result of recent polyploidization events forming the basis for separation of new species (possible in *P. hazenii*, because the two investigated individuals originate from the same geographic region). More collections covering the whole distribution areas of the above-mentioned species need to be investigated to get a clearer picture of the implications of the reported differences in ploidy levels.

As already assumed for *Psychotria* subgen. *Psychotria* in the Pacific (Kiehn & Lorence, 1996), a predisposition for polyploidization could also be an important factor for comparatively rapid speciation in the Neotropical Palicoureeae, notably in *Psychotria* subgen. *Heteropsychotria*. The much higher number of Neotropical species in the Palicoureeae compared to those in the Psychotrieae in the Neotropics thus might be the result of a higher polyploidization capacity (as trigger for speciation) in the former tribe. As Table 2 shows, the frequency of ploidy levels differs considerably for the subgenera of *Psychotria*. Although in Neotropical members of *Psychotria* subgen. *Psychotria*, 70% of the species counted so far are diploids, *Psychotria* subgen. *Heteropsychotria* has a very high number of polyploids (nearly 65% of the counted species). The assumption of a relationship between frequency of polyploidization and number of species could even be valid for the whole Palicoureeae, as the percentage of polyploids in this assemblage is more than 60% (Kiehn, unpublished data).

RUBIIDINAE I SENSU ROBBRECHT AND MANEN (2006)

Anthospermeae. The only count from this tribe refers to a member of the genus *Nertera* Banks & Sol. ex Gaertn. The proposed inclusion of this genus into *Coprosma* J. R. Forst. & G. Forst. (Heads, 1996) is not supported by molecular studies (Anderson et al., 2001; Markey et al., 2004) and is, therefore, not followed in the present paper. The count for *N. depressa* Banks & Sol. ex Gaertn. revealed the same result ($2n = 44$) as all earlier-counted collections of this widespread species (see survey in Kiehn, 1996; Kiehn et al., 2005).

Rubieae. Members of two genera of this tribe (*Didymaea* Hook. f. and *Galium* L.) were investigated in the current study.

In *Didymaea*, the first count in the genus (for *D. mexicana* Hook. f.) revealed $2n = 22$. By far the most frequent number in this tribe is $x = n = 11$, thus the result is not informative with regard to the taxonomic position of the genus. It does not, however, contradict a basal position of the genus within the Rubieae as indicated by molecular studies (Natali et al., 1996).

The investigated *Galium* species is *G. hypocarpium* (L.) Endl. ex Griseb., a species with a large distribution area from Central America to the southern part of South America and a member of the group formerly treated as the genus *Relbunium* Benth. & Hook. f. (Dempster, 1990). Three species of this *Relbunium* group have been counted so far: *G. araucanum* Phil., *G. hirsutum* Ruiz & Pav., and *G. hypocarpium* (L.) Endl. ex Griseb. *Galium araucanum* from southern Chile is diploid (Kiehn et al., 2005). Two ploidy levels are reported for *G. hirsutum* from Peru: diploidy (Huynh, 1965) and tetraploidy (Diers, 1961). For *G. hypocarpium*, there are three different ploidy levels known: diploidy ($n = 11$: Kiehn et al., 2005) from southern Chile, tetraploidy ($2n = 44$) reported here for a population from Costa Rica, and hexaploidy ($n = 33$: Huynh, 1965) from Peru. These results support the statement of Dempster (1990) in the context of the *Relbunium* group of *Galium*: "...since the counting of chromosomes has been of great taxonomic value with the North American species of *Galium*...it would almost certainly be valuable for the South American species as well." When comparing the morphology of the investigated collections of *G. hypocarpium* from Costa Rica and Chile, the Costa Rican plants ($4x$) are much stouter and more robust than the plants from Chile ($2x$). Thus, in addition to chromosome counts, detailed morphological and molecular studies also seem to be worthwhile in this group.

RUBIIDINAE II SENSU ROBBRECHT AND MANEN (2006)

Spermaceae s.l. (including *Hedyotideae* p.p. and *Manettieae*). In recent years, several papers based on molecular data provided evidence for the Spermaceae s. str. (as, e.g., defined in Robbrecht, 1993) being nested within members of the traditionally circumscribed Hedyotideae (see survey by Robbrecht & Manen, 2006). The correct name for the emended tribe is Spermaceae (Bremer, 1996; Andersson & Rova, 1999; Bremer & Manen, 2000). This tribe is the sister group to the Paleotropical Knoxieae s.l. (Robbrecht & Manen, 2006).

The presented new chromosome data further corroborate the predominance of $x = 14$ in the clade of the Spermaceae s. str. They include first chromosome number reports for the genera *Emmeorrhiza* Pohl ex Endl. and *Hemidiodia* K. Schum. (a genus included in *Borreria* G. Mey. or *Spermaceae* L. by some authors, see, e.g., Bacigalupo & Cabral, 1996), and for species of the genera *Diodia* L. s. str., *Ernodea* Sw., and *Spermaceae* (including *Borreria*). In the traditionally circumscribed Hedyotideae, new counts could be obtained for species of the genera *Arcytophyllum* Roem. & Schult., *Bowardia* Salisb., and *Manettia* Mutis ex L. In *Arcytophyllum*, the new data indicate tetraploidy on $x = 9$. These results are in accord with the two earlier counts for the genus, both for *A. thymifolium* (Ruiz & Pav.) Standl., for which Diers (1961) reported $2n = \text{ca. } 30$ and Lewis (1963: 17) $2n = 36$. In *Bowardia*, different ploidy levels ($4x$, $8x$, and $12x$) on $x = 9$ are now documented for *B. ternifolia* (Cav.) Schltdl. (see also Lewis, 1962). Further studies in this species seem worthwhile to investigate potential correlations of geographic or altitudinal distribution pattern of *B. ternifolia* with the different ploidy levels. In *Manettia*, $x = 11$ has been established as the basic number for two species. Also, the existence of the diploid level is clearly documented now (for *M. barbata* Oerst.).

Kiehn (1986, 1995) presented and discussed possible models for the origin of the predominant basic number $x = 14$ observed in the clade of the Spermaceae s. str. All these models interpret $x = 14$ as a paleotetraploid state derived from a basic number existing in Hedyotideae. Chromosome numbers reported for the Neotropical genus *Galianthe* Griseb. ($2n = 32$ and $2n = 30$, see Daviña & Cabral, 1991; Kiehn, 1995) could be indicative for such a derivation: a descending dysploidy from a potential tetraploid ancestor exhibiting a basic number of $x = 9$. Unpublished molecular studies on the Spermaceae s.l. (Dessein, pers. comm.) could be helpful in the context of this discussion. They suggest a position of *Galianthe* either within or at the base of the

Spermaceae s. str. and indicate that *Manettia* and *Bouvardia* often fall in a clade together with the Spermaceae s. str. The presence of taxa with basic numbers higher than $x = 7$ found in close proximity to or within the Spermaceae s. str. gives further support for the hypothesis of $x = 14$ being the result of a descending dysploid series.

CONCLUSION

The reported data clearly show the significance of chromosome data in defining relationships on the species, genus, or even tribal level, especially when combined with the results of molecular studies. Despite the considerable increase of karyological knowledge about Neotropical Rubiaceae–Rubioidae in this paper, there are still huge gaps of knowledge remaining. Aside from adding data for genera not studied yet or insufficiently known (e.g., *Rudgea*), further studies should be preferably related to taxa such as *Bouvardia*, where the existing data indicate that new counts, in combination with molecular, morphological, and spatial distribution pattern analyses, can provide valuable contributions to an understanding of speciation and radiation.

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- BOLIVIA.** Near Cochabamba, seeds received from H. Billensteiner (Palmengarten Frankfurt), cult. in HBV sub RR 1028, *Kiehn RR 1028* (WU).
- BRAZIL.** s. loc., cult. in HBV sub RR-90-15, *Forstreuter s.n.* (WU); NW Parana, in der Nähe von Fao Matheus, ± 250 m, cult. in HBV sub RR 87-70, *Lindeman JCL 599* (WU). **Amazonas:** near Manaus, *Ehrendorfer 74913-1-6* (DUKE, WU), *Ehrendorfer 74913-6-3* (DUKE, WU), *Ehrendorfer 74915-1-16* (WU), *Ehrendorfer 74915-1-17* (WU), *Ehrendorfer 74922-1-4* (WU), *Ehrendorfer 74922-1-5* (DUKE, WU), *Ehrendorfer 74922-1-6* (DUKE, WU), *Ehrendorfer 74922-2-9* (WU); N of Manaus, Km 29 on Itacoatiara Rd., Ceplac Cacao Station, *Ehrendorfer 74919-2-1* (WU), *Ehrendorfer 74927-1-13* (WU). **Bahia:** W von Correntina, bei Veredãozinho (Dona Amelia) *Gottsberger 11-1267* (WU), *Gottsberger 13-2267* (WU), *Gottsberger 15-2267* (WU), *Gottsberger 16-2267* (WU). **Guanabara [Rio de Janeiro]:** Rio de Janeiro, Pedra de Cávea, W side of mtn. above Tijucamar to Praca de Bandeira, *Ehrendorfer 7300-4003* (WU), *Ehrendorfer 7300-4004* (WU). **Pará:** Campinaraña do Palha near Belem, *Ehrendorfer 7300-4208* (WU). **São Paulo:** Botucatu, Rubião Junior, Mata de Butignolli, *Ehrendorfer & Gottsberger 73821-3-5* (WU); São Paulo-City, Parque do Estado, *Gottsberger 15-241066* (WU), *Gottsberger 18-241066* (WU); Serra Do Mar, ca. 60 km SE São Paulo, Paranapiacaba Nat. Res., *Ehrendorfer & Gottsberger 7300-1312* (WU), *Ehrendorfer & Gottsberger 73822-602* (WU), *Ehrendorfer & Gottsberger 73825-1312* (WU), *Ehrendorfer & Gottsberger 73825-1319* (WU), *Ehrendorfer & Gottsberger 73825-1324* (WU).
- CARIBBEAN REGION.** s. loc., seeds from HB Bonn (1983-1104), cult. in HBV sub RR 756, *Kiehn RR 756* (WU).
- CHILE. Region III [Atacama]:** Provincia de Huasco, Estación Chacritas N Vallenar, *Grau 2522* (Herb. Grau, M. WU).
- COSTA RICA. Alajuela:** along rd. down from Poas Volcano, ca. 2 km outside of Natl. Park, *Kiehn et al. MK 920211-2/2* (WU); betw. Poasito & Poas, *Crex 9906-1* (MO). **Cartago:** ca. 1 km N Finca Cristina near La Suiza, *Kiehn et al. MK 880321-1/3* (MO, WU), *Kiehn et al. MK 880321-1/4* (MO, SJ, WU), *Kiehn et al. MK 880321-1/5* (MO, SJ, WU); ca. 1–1.5 km (Luftlinie) östl. von Sitio de Mata (= ca. 3.5 km N von La Suiza), *Kiehn & Kiehn MK 090886-2/3* (WU), *Kiehn & Kiehn MK 090886-2/6* (MO, WU); Gelände des CATIE bei Turrialba, *Kiehn & Kiehn MK-050886-1/2* (MO, WU), *Kiehn & Kiehn MK 270786-1/1* (MO, SJ); Gelände des CATIE, Regenwald im Tal des Río Reventazon, *Kiehn & Kiehn MK 190385-2/1* (DUKE, US, WU), *Kiehn & Kiehn MK 200385-2/1* (WU), *Kiehn & Kiehn MK 200385-2/5* (DUKE, WU); Guayabo historical site, cult. in HBV sub RR 91-21, *Till & Till s.n.* (WU); Hazienda Florida Sur bei Turrialba, Gebiet der Lá Montaña-Versuchsplantage, *Kiehn & Kiehn MK 300786-1/3* (WU), *Kiehn & Kiehn MK 300786-1/5* (WU); Km 84.5 on "Ruta 2" (Cartago-San Isidro del General, "Interamericana"), near Cerro Zacarales, *Kiehn & Kiehn MK 880311-3/1* (SJ, WU); Straße Turrialba-Lá Suiza, ca. 1 km vom CATIE (Instituto Interamericana de Ciencias Agrícolas) entfernt in Richtung Lá Suiza, kurz vor der Brücke über den Río Reventazon, *Kiehn & Kiehn MK 260786-1/1* (WU); Tapantí Refugio Nat. de Fauna Silvestre, near to the border of Río Grande de Orosi, *Kiehn & Kiehn MK 880319-1/2* (MO, SJ, WU), *Kiehn & Kiehn MK 880319-1/8* (MO, WU); Viehweide südl. des letzten Hauses von San Antonio, *Kiehn & Kiehn MK 210385-1/9* (SJ). **Heredia:** Braulio Carrillo Natl. Park, Estacion Carrillo at new rd. betw. San José & Guapiles, *Kiehn & Kiehn MK 880309-1/1* (MO, SJ, WU), *Kiehn & Kiehn MK 880309-1/3* (MO, WU), *Kiehn & Kiehn*

APPENDIX 1. Collection data for the investigated plants.

ARGENTINA. s. loc., seeds received from HB Carlos Eralio R. Ruiz. Buenos Aires (Kat. 1987-126), cult. in HBV sub RR-88-18, *Kiehn RR-88-18* (WU).

BAHAMAS. Great Abaco, fixation received from V. Negrón-Ortiz, *Negrón-Ortiz s.n.* (1) (MU), *Negrón-Ortiz s.n.* (2) (MU); Great Abaco, South Side Dock trail, *Negrón-Ortiz & Pinder 335, 336* (MU); Great Inagua, fixation received from V. Negrón-Ortiz, *Negrón-Ortiz s.n.* (3) (MU).

MX 880309-1/4 (SJ, WU), *Kiehn & Kiehn* MK 880309-1/7 (MO, SJ, WU), *Kiehn & Kiehn* MK 880309-1/11 (WU); Braulio Carrillo Natl. Park, trail behind MINAREM station, *Crex* 9907-2 (MO); zwischen Porrosati und Sacramento, ca. 4 km vor Beginn des Natl. Park Braulio Carrillo am Fuß des Vulkan Barva, *Kiehn & Kiehn* MK 030485-1/1 (DUKE, WU); San Pablo de Heredia, *Kiehn & Kiehn* MK 160385-1/1 (WU).

Limón: Cahuita, Ortsgebiet, *Kiehn & Kiehn* MK 250385-1/2 (WU); Tortuguero Natl. Park, base of Tortuguero peak, *Crex* 9909-2 (WU), *Heiselmayer* 950212-1/1 (WU). **Puntarenas:** Gemeinde Garabito, Ortsgebiet von Jaco, *Kiehn & Kiehn* MK 290385-1/2 (WU); Monteverde Biol. Res., cult. in HBV sub RR-90-21, *Frimmel* 8-90 (WU), *Kiehn & Kiehn* MK 880316-1/3 (MO, SJ, WU), *Kiehn & Kiehn* MK 880316-1/4 (MO, WU); Monteverde Cloud Forest Preserve, Sendero Bosque Nuboso, *Heiselmayer* 950217-1/1 (WU), *Kiehn* MK 950217-1/2 (WU); region Golfito, ca. 2 km E of La Gamba (Esquinas), *Heiselmayer* 950220-2/1 (MO), *Huber & Weißenhofer* 9917-1 (MO, WU); region Golfito, ca. 2 km E of La Gamba, Parque Nac. Piedras Blancas, Sección "Esquinas," Bosque de los Austriacos, *Bernhard & Greger* HG2607083 (WU), *Bernhard & Greger* HG2607086 (WU), *Kiehn* MK 961117-1/2 (INB), *Kiehn* MK 961117-1/4 (INB, WU), *Kiehn* MK 961117-1/6 (INB), *Kiehn* MK 961118-2/1 (INB, WU), *Kiehn* MK 961118-4/1 (INB, WU), *Kiehn* MK 961118-4/2 (INB, WU), *Kiehn* MK 961119-2/1 (INB, WU), *Kiehn* MK 961119-3/6 (INB, WU); below entrance of Rincón de la Vieja Natl. Park, *Heiselmayer* 950215-3/1 (MO); Rincón de la Vieja Natl. Park, near summit, *Crex* 9913-4 (WU); zwischen Pto. Quepos und Manuel Antonio, Umgebung des Nationalparks Manuel Antonio, *Kiehn & Kiehn* MK 200886-1/1 (WU). **San José:** Braulio Carrillo Natl. Park, Valle Virgen, *Crex* 9915-2 (MO); nahe von Alto La Palma, bei Lecheria Alto La Palma und Finca Cumbres, *Kiehn & Kiehn* MK 030485-3/3 (WU), *Kiehn & Kiehn* MK-030485-3/5 (WU); near Zurqui at new rd. betw. San José & Guapiles, just outside Braulio Carrillo Natl. Park, *Kiehn & Kiehn* MK 880309-2/1 (MO, SJ, WU), *Kiehn & Kiehn* MK 880309-2/2 (WU), *Kiehn & Kiehn* MK 880309-2/5 (SJ, WU), *Kiehn & Kiehn* MK 880331-1/1 (SJ).

CUBA. s. loc., seeds from HB La Habana (Cuba) 1984-1051, cult. in HBV, *Kiehn* 1984-1051 (WU); s. loc., seeds from HB La Habana (Cuba) 1984-1028, cult. in HBV sub RR 87-21, *Kiehn* RR-87-21 (WU).

DOMINICAN REPUBLIC. **El Seibo:** Arroyo Las Cabirmas, 5 km S of Miches to Nisibon hwy., on rd. to Las Cabirmas, cult. in HBV sub RR-87-81, *Zanoni et al.* 15980 (WU); Eastern Cordillera betw. Pedro Sanchez & Miches, *Till* 18017 (WU).

FRENCH GUIANA. Rte. N2, Km 50, cult. in HB Meise sub no. 83-2784, *Billiet* 1860 (BR).

MEXICO. s. loc., cult. in HB Kew sub no. 38-91201, *Balls* 5465 (K); native in the Botanic Garden UNAM, Mexico City, cult. in UCBC sub No. 75-665, cult. in HBV sub RR-916, *Boutin* 3956 (WU); La Joya on rd. from Jalapa to Mexico City, cult. in HB Kew sub no. 78-01766, *Beyer* 166 (K); "Pedregal" close to the University Campus, Mexico City, *Vogel et al.* 1984-2-12/2 (DUKE, WU); Tuxtepec, seeds from HB Göttingen 1986-1602, cult. in HBV, *Kiehn* 1986-1602 (WU). **Chiapas:** "Lagunas de Montebello" Natl. Park, near Lago Bosque Azul, *Vogel et al.* 1984-87-2/1 (DUKE, WU); Parque Educativo "Laguna Bélgica," ca. 20 km N of Ocozacoautla (ca. 30 km W of Tuxtla Gutiérrez), *Vogel et al.* 1984-81-10/2 (WU). **Morelos:** Malinalco, nahe Ixtapan, *Ehrendorfer* 7900-57-1 (WU). **Oaxaca:** Vera Cruz, Valle Nacional, *Ehrendorfer* 7900-46-1 (WU).

PANAMA. Canal Zone, Barro Colorado Island: Barbour-Lathrop Trail, W & NW of Smithsonian Laboratory, *Ehrendorfer* 6400-2502 (DUKE, WU); Canal Zone, Barro Colorado Island: Wheeler-Armour Trail to Big Trees, *Ehrendorfer* 6400-2903 (WU).

PUERTO RICO. **Ponce:** NE of Ponce on rte. 139 at Km 23.8, *Taylor* 7084 (DUKE), *Taylor* 7087 (DUKE, WU). **Ponce-Jayuya:** on Cerro Punta, *Taylor* 7076 (DUKE), *Taylor* 7077 (DUKE, WU). **Utuaño:** *Taylor* 6935 (DUKE), *Taylor* 6938 (DUKE).

U.S.A. Florida: s. loc., 24 Mar. 1973, cult. in HBV sub RR 87-74, *Eckhardt* s.n. (WU).

VENEZUELA. **Aragua:** NW of Maracay, Parque Nac. Rancho Grande, surroundings of Biological Station near Portachuelo Pass, *Ehrendorfer* 6400-5409 (DUKE, WU).

Bolívar: Km 116 along the rd. from Santa Elena de Uairén to El Dorado, near La Escalera, at Salto El Danto, *Till* 16058 (WU), *Till* 16059 (WU); near Canaima, *Ehrendorfer* 74104-1-9 (WU); cult. in HBV sub RR-1084-9, *Ehrendorfer* 74108-4-9 (WU); Quebrada Jaspe (Koko Parí) along the rd. from Sta. Elena de Uairén to El Dorado, *Till* 16015 (WU); Santa Elena de Uairén, near the Ya-Koo hotel, *Till* 16063 (WU). **Mérida:** Mtns. N of Mérida, Cerro de las Flores, *Ehrendorfer* 6400-4003 (DUKE, WU); Teleferico Pico Espejo above Mérida, surroundings of Station II, La Montana, *Ehrendorfer* 6400-5005 (DUKE, WU), *Ehrendorfer* 6400-5009 (WU).

s. loc.: seeds from HB Basel 1983-1499, *Kiehn* 1983-1499 (WU); seeds from HB Basel 1983-1501, *Kiehn* 1983-1501 (WU); seeds from HB Bonn 1983-1105, *Kiehn* 1983-1105 (WU); seeds from HB Hauensis (Copenhagen) 1982-1706, cult. in HBV sub RR 663, *Kiehn* RR 663 (WU); cult. in HB Kew ex HB Oxford (No 77-00377), cult. in HBV sub RR 95-04, *Kiehn* RR 95-04 (WU).